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The specific nutrient synergy and their effect on the reduction of pathogens resistance to antibiotics

Monika Sienkiewicz¹*, Edward Kowalczyk², Mateusz Kowalczyk³, Katarzyna Kozak³, Maciej Glowacki⁴ and Anna Glowacka¹

¹Environmental Biology Department, Medical University of Lodz, ul Żeligowskiego 7/9, 90-752 Lodz, Poland.
²Pharmacology and Toxicology Department, Medical University of Lodz, ul Żeligowskiego 7/9, 90-752 Lodz, Poland.
³Military Medical Faculty, Medical University of Lodz, ul Żeligowskiego 7/9, 90-752 Lodz, Poland.
⁴PHARMGLO SP. J. Glowaccy, 95-020 Andrespol ul. Rokicińska 124, Poland.

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The high level of pathogens resistance is becoming a huge problem in the health care system. The difficulty to treat recurrent infections also contributes to the spread of resistant microorganisms in the environment. The antibacterial therapy is very often not effective and costly. On the other hand, there is a growing interest in using natural plant components as effective antibacterial agents and valuable complement to the anti-infective therapy. The aim of this study was to determine the modulation of bacterial resistance by Epi-Quercican™, a synergistic combination of specific plant extracts, amino acids, minerals and vitamins. The Gram-positive and negative clinical strains isolated from several clinical materials, hospital equipment and environment were cultivated in the brain heart infusion broth in absence and presence of Epi-Quercican™ at 37°C for 2 h. In both cases, the sensitivity to antibiotics was tested by the disc-diffusion method. Epi-Quercican™ was efficacious against the tested pathogens and significantly reduced to the level of their resistance. The results suggest that the nutrient synergy contained in Epi-Quercican™ can be utilized in the treatment of infectious diseases.

Key words: Multidrug resistance, Epi-Quercican™, Staphylococcus aureus, Enterococcus faecalis, Escherichia coli, Enterobacter cloacae, Acinetobacter baumannii.

INTRODUCTION

It has been found that after a stormy bloom of medicine related to synthetic drugs, means of natural origin are used more often. For instance most of the Brazilian population (80%) consumes only 37% of the commercially available drugs and depend almost exclusively on medicines of natural origin (Funari and Ferro, 2010). This country has been famous for the world’s highest biodiversity, accounting for over 20% of the total number of known species. Each geographic region is abundant in many valuable medicinal plants which have long been

*Corresponding author. E-mail: monika.sienkiewicz@umed.lodz.pl.

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used in folk medicine. A lot of them have been described in native pharmacopeia. However, thanks to areas such as systematic botany, phytochemistry, phytopharmacology or biotechnology, the properties of various active plant metabolites are becoming increasingly known. Drugs of natural origin are becoming more popular because it was noticed very quickly that synthetic drugs have numerous side effects which are often difficult to predict, require a complex manufacturing process and are expensive. Moreover, a huge problem with infections caused by multidrug resistant pathogens calls for the continuous search for effective antimicrobial agents. The necessity of researching new medications is largely the result of microorganisms ability to develop different mecha-nisms of resistance to the recommended and commonly used synthetic drugs. Among them are multidrug resistant strains of staphylococci as methicillin-resistant *Staphylococcus aureus*- MRSA, methicillin-resistant *Staphylococcus epidermidis*- MRSE and other coagulase-negative staphylococci- MR-CNS, enterococci resistance to aminoglycosides (HLAR) and glycopeptide (VRE) and urinary *Staphylococcus* clinical strains were identified according to standard methods of culturing on Columbia Agar (bioMerieux), on Mannitol Salt Agar (bioMerieux), and determining the ability of bacteria to produce catalase and coagulase (bioMerieux) and using API Staph tests (bioMerieux). Enterococci were identified to the genus level on Columbia Agar (bioMerieux), Enterococcus sol Agar (Emapol) and using API 20 Strep tests (bioMerieux). Gram-negative bacteria were cultured with use of Columbia Agar (bioMerieux) and Mac Conkey Agar (bioMerieux). They were identified to the species level with the use of API 20 E and API 20 NE tests (bioMerieux) according to manufacturer's instructions. The bacteria were incubated at 37°C for 24 h.

Epi-Quercicantm is composed of the following ingredients in the relative amounts indicated: 125.8 mg of vitamin C (as ascorbic acid, Mg, Ca and palmitate ascorbate), 166.7 mg of L-lysine, 125 mg of L-proline, 83.3 mg of L-arginine, 33.3 mg of N-acetyl L-cysteine, 166.7 mg of standardized green tea extract (80% polyphenols), 8.3 mg of quercetin, 5 µg of selenium, 333 µg of copper and 167 µg of manganese.

**Bacterial growth conditions**

All isolates of *S. aureus*, *E. faecalis*, *E. coli*, *E. cloacae*, *A. baumannii* were grown in 5 ml brain heart infusion at 37°C for 18 h. A total of 100 µl of culture were added to 5 ml of brain heart infusion broth containing Epi-Quercican™ at 25 and 50 mg/ml. Both concentrations were used for the tests of reducing microbial resistance. The mixtures were incubated in a 37°C shaker. The change in the resistance pattern was calculated and expressed in percent.

**Bacterial susceptibility to antibiotics**

Bacterial cultures of the tested strains on Columbia Agar medium at 37°C for 24 h were performed. Inoculum and optical density of 0.5MF (bioMerieux densimeter) was applied on Mueller-Hinton II Agar (bioMerieux) and incubated at 37°C for 18 h. Susceptibility testing was carried out with the use of the disk-diffusion method with the following antibiotics (Becton Dickinson) used against all tested *S. aureus* and *E. faecalis* clinical strains: GM- gentamicin (10 μg), CIP- ciprofloxacin (5 μg), C- chloramphenicol (30 μg), TEC- teikoplanin (30 μg), FOX- cefoxitin (30 μg), CIP- ciprofloxacin (5 μg), FOX- cefoxitin (30 μg), NET-netilmicin (30 μg), TOB- tobramycin (10 μg), SXT- trimethoprim/sulfamethoxazole (1.25 μg/23.75 μg), FOX- cefoxitin (30 μg), E- erythromycin (15 μg), DA- clindamycin (2 μg), SXT- trimethoprim/sulfamethoxazole (1.25 μg/23.75 μg), FOX- cefoxitin (30 μg), E- erythromycin (15 μg), DA- clindamycin (2 μg), RA- rifampicin (5 μg), LQZ- linezolid (30 μg), FD- fusidic acid (10 μg), QQ- quinupristin/dalfopristin (15 μg), K- kanamycin (30 μg), MUP- mupirocin (200 μg); and only for *E. faecalis* strains: RA- rifampicin (5 μg), LQZ- linezolid (30 μg), P- penicillin 10 IU, AM- ampicillin (10 μg), TEC- teikoplanin (30 μg), N/F- nitrofurantoin (300 μg), NOR-
norfloxacin (10 μg), FOS- fosfomycin (200 μg), S- streptomycin (300 μg). For all clinical strains of *E. coli, E. cloacae* and *A. baumannii* were used: GM- gentamicin (10 μg), PIP- pipercillin (100 μg), TIC- ticarcillin (75 μg), TZP- piperacillin/tazobactam (100/10 μg), TIM- ticarcillin/clavulanic acid (75 μg /10 μg), CTX- cefotaxim (30 μg), CAZ- cefazolin (30 μg), FEP- cefepime (30 μg), ATM- aztreonam (30 μg), IPM- imipenem (10 μg), MEM- meropenem (10 μg), ETM- ertapenem (10 μg), DOR- doripenem (10 μg), CIP- ciprofloxacin (5 μg), AN- amikacin (30 μg), NET- netilmicin (30 μg), TOB- tobramycin (10 μg), C- chloramphenicol (30 μg) and SXT- trimethoprim/sulfamethoxazole (1.25 μg/23.75 μg); for clinical strains of *E. coli* and *Enterobacter cloacae*: CXM- cefuroxim (30 μg), TE- tetracycline (30 μg), TGC- tigecyclin (15 μg), only for *E. coli* strains: AMC- amoxicillin / clavulanic acid (20 μg /10 μg), CF- cefalotin (30 μg), CZ- cefazolin (30 μg), AM- ampicillin (10 μg), FOX- cefoxitin (30 μg); and only for *A. baumannii* strains: SAM- ampicillin/sulbactam (10/10 μg) and CL - colistin (50 μg).

*S. aureus* ATCC 29213, *E. faecalis* Van B ATCC 51299, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922 and *A. baumannii* ATCC 19606 strains were used as a control.

The results were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines.

**RESULTS**

The bacterial resistance to antibiotics

*S. aureus* strains isolated from wounds, ulcers, bronchial secretions, abdominal cavity exudates and blood were resistant to: E (100%); K (90%); DA (70%); CIP- AN (60%); TOB, C, FOX, MUP (40%); GM, TE, RA, LNZ (30%); FD, QD (20%); SXT (10%); VA and TGC (0%). *S. aureus* strains isolated from wounds, ulcers, bronchial secretions, abdominal cavity, wounds, urine, hospital equipment, and hospital environment showed resistance to: GE (100%); TE (90%); S (80%); P, AM (70%); C (60%); CIP (50%); NOR (50%); TGC (40%); RA, LNZ (30%); F/N (10%); and VA, TEC, FOS (0%). The resistance of *E. coli* isolates was as follows: AMC, AM, PIP, TIC, TE, SXT (90%); TIM (80%); C (70%); CIP, NET, CXM, AN (60%); CZ, CAZ, FEP, GM, TZP, ATM (50%); CTX (20%); CF, IPM, TGC, TOB (10%); FOX, MEM, ETP, DOR (0%).

*Enterobacter cloacae* strains isolated from abdominal cavity, wounds, ulcers, bronchial secretion, urine, blood and hospital environment were resistant to: GM, TOB, CXM (90%); PIP, TIC, SXT (80%); CAZ, TE (70%); CTX, CIP (50%); ATM, AN (40%); FEP, NET, C (30%); TZP, TIM (20%); TGC (10%); IPM, MEM, ETP, DOR (0%). Tested strains from *A. baumannii* genera were characterized as the most resistant to recommended antibiotics. As such, isolates from bronchial secretion, wounds, urine, sputum, anus, and hospital environment were 100% resistant to: PIP, TZP, CTX, CIP, TOB, C, SXT and 90% to: Tic, TIM, CAZ, FEP, AN, NET. 50% of tested strains were resistant to carbapenems: IPM, MEM, ETP and DOR. Only colistin was active against all *A. baumannii* isolates.

The reduction of tested bacterial strains resistance by Epi-Quercican™

The effect of Epi-Quercican™ on the microbial resistance profile of the tested bacterial strains was concentration dependent. Reduction of bacterial resistance was most pronounced at Epi-Quercican™ concentrations of 50 mg/ml and after 2 h of incubation. The majority of bacterial strains from *S. aureus* genera were resistant to E and K. In the presence of Epi-Quercican™, the resistance to these antibiotics was reduced from 100 to 60% and from 90 to 60%, respectively. In addition, the significant decrease of resistance to C (from 40 to 10%), RA and LNZ (from 30 to 10%) was observed. Epi-Quercican™, however, did not affect the resistance of *S. aureus* tested clinical strains to CIP. The modulation of microbial resistance of *S. aureus* isolates by Epi-Quercican™ is presented in Figure 1.

Epi-Quercican™ did not have major effect on the sensitivity improvement of *E. faecalis* clinical isolates to GM (change from 100 to 90%) and similarly to TE, S, C with the reduction of resistance by about 20%. After exposure to Epi-Quercican™ a larger number of enterococci isolates displayed higher sensitivity to TGC, RA and LNZ. No change in the level of resistance to P and AM was observed. The modulation of microbial resistance of *E. faecalis* isolates by Epi-Quercican™ is presented in Figure 2. The greatest effect of resistance modulation by Epi-Quercican™ in *E. coli* clinical strains was seen against TIC, TE, CIP, NET, CXM, GM, TZP, ATM resulting in lowering the resistance to these antibiotics by about 20%. There was no increase in sensitivity against PIP and SXT. The modulation of microbial resistance of *E. coli* isolates by Epi-Quercican™ is presented in Figure 3. *E. cloacae* clinical isolates exposed to Epi-Quercican™ become more sensitive to CAZ with the reduction of resistance by about 30% and to CXM, TE (reduction by about 20%). For these genera of bacteria the sensitivity to other antibiotics, such as GM, SXT, CIP, AN and C was unaffected by Epi-Quercican™. The modulation of microbial resistance of *E. cloacae* isolates by Epi-Quercican™ is presented in Figure 4. The modulating properties of Epi-Quercican™ in reducing the level of antibiotic resistance of *A. baumannii* strains were mainly seen for PIP, TZP, CTX, TOB, SXT and ATM. In this case, the resistance to antibiotics was decreased by about 20%. The resistance to antibiotics: CIP, C, CAZ, FEP and AN was not affected.

The modulation of microbial resistance of *A. baumannii* isolates by Epi-Quercican™ is presented in Figure 5.

**DISCUSSION**

In general, the sensitivity of all tested clinical strains to most recommended antibiotics has significantly improved in the presence of Epi-Quercican™. The results of
Figure 1. Modulation of microbial resistance of *S. aureus* isolates by Epi-Quercican™.

Figure 2. Modulation of microbial resistance of *E. faecalis* isolates by Epi-Quercican™.

Figure 3. Modulation of microbial resistance of *E. coli* isolates by Epi-Quercican™.
susceptibility testing show that for the S. aureus clinical strains the sensitivity to E. K and DA significantly increased after Epi-Quercican™ treatment. We found that Epi-Quercican™ was effective in reducing E. faecalis resistance to GM, TE and S. Exposure to Epi-Quercican™ decreased E. coli resistance to most tested antibiotics as: Tic, TE, CIP, NET, CXM, GM, TZP and ATM. The E. cloacea strains susceptibility was lower for several antibiotics (20 to 30% range), in particular to CAZ (50% decrease in resistance). The most resistant pathogens- tested A. baumannii clinical strains were the least sensitive to Epi-Quercican™ modulating activity, but the important fact was the significant reduction of resistance to carbapenems, mainly to ATM. Our study demonstrates that Epi-Quercican™ can be used to modulate pathogens resistance to various antibiotics. We observed that the change of susceptibility to antibiotics depends on the level of resistance and the genus of bacteria. Earlier study by Harakeh et al. (2013) on modulation of the antimicrobial resistance by Epi-Quercican™ in bacteria isolated from dairy products exposed to select antibiotics showed the highest percentage increase in antimicrobial susceptibility of E. coli, Salmonella sp. and Yersinia sp. for gentamicin followed by cefotaxime (Harakeh et al., 2013). The percentage increase in susceptibility to clindamycin in the
The presence of Epi-Quercican™ was not significant in *S. aureus*, but it was significant in *L. monocytogenes*. The increase of *L. monocytogenes* susceptibility to gentamicin was not significant.

In our study, we observed that Epi-Quercican™ exposure resulted in the highest increase in susceptibility to gentamicin for *E. coli* isolates, but not for *E. cloacae*.

An interesting study on the use of dietary supplements, such as ascorbic acid with antibiotics in treating infectious diseases was conducted by Abbas (2012). He investigated the synergy between antibiotics and each of N-acetylcysteine, ambroxol and ascorbic acid against *P. aeruginosa* clinical strains. His study showed a synergistic effect of N-acetylcysteine with β-lactam antibiotics, tetracycline in 100% of isolates, with chloramphenicol in 80% and with gentamicin in 60% of isolates. The antagonism was observed with gentamicin in 20% of isolates. Combinations of N-acetylcysteine and ambroxol showed the highest synergy with each of cefepime, ceftazidime, cepofarzepone and meropenem and those of tetracycline. The synergy for ascorbic acid was found with chloramphenicol in 60% of isolates and with meropenem, cefepime and cepofarzepone in 20% of *P. aeruginosa* isolates. The combination of ascorbic acid with ceftazidime, levofloxacin, gentamicin and tetracycline showed the synergistic effect in all bacterial isolates (100%), with cepofarzepone, cefepime and meropenem in 80% and with chloramphenicol in 40% of isolates. One of the major constituents of Epi-Quercican™ is epigallocatechin gallate which according to Kurinčič at al. has a good modulatory activity over the extrusion across the outer membrane of the macrolides such as erythromycin, azithromycin, clarithromycin, dirithromycin and tyllosin, both in sensitive and resistant *Campylobacter* isolates (Kurinčič et al., 2012). According to these authors, EGC uncodes *Campylobacter* multidrug efflux systems and thus could have an impact on restoring macrolide efficacy in resistant strains. Stapleton et al. (2004) proved that aqueous extracts of Japanese green tea (*Camellia sinensis*) are able to reverse beta-lactam resistance in methicillin-resistant MRSA. Minimum inhibitory concentration (MIC) values for oxacillin were reduced from 256 and 512 to 1-4 mg/l, respectively, in the presence of these polyphenols. The modulation of beta-lactam resistance by ECG significantly enhanced the activities of fluoxacillin and the carbapenem anti-biotics imipenem and meropenem against MRSA isolates. According to Silva and Fernandes Júnior (2010) review article, the mechanisms of antimicrobial activity of natural compounds can be diverse, such as involving disintegration of the cytoplasmic membrane, destabilization of the proton motive force (PMF), electron flow, active transport and coagulation of the cell content. In the case of epicatechins, the mechanism of action is strictly connected with the disruption of membrane function (Silva and Fernandes Júnior, 2010). The impact of active plant metabolites on the bacterial cells is mainly dependent on the differences in the structure of Gram-positive and negative bacteria cell walls. It is known, that in the case of Gram-negative bacteria, the outer membrane disintegration can release the lipopolysaccharide (LPS) increasing permeability of the cytoplasmic membrane. These factors can also affect the degree and characteristics of modulation of bacterial resistance to antibiotics by natural plant components.

Conflicts of Interests

The authors have not declared any conflict of interests.

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References


European Committee on Antimicrobial Susceptibility Testing (EUCAST). Breakpoint Tables for Interpretation of MICs and Zone Diameters, version 2.0; valid from 1 January 2012; Available online: www.eucast.org (accessed on 16 August 2012).


